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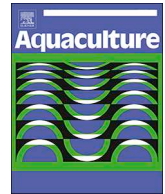
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# Mechanical shock during egg de-adhesion and post-ovulatory ageing contribute to spontaneous autopolyploidy in white sturgeon culture (*Acipenser transmontanus*)

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## ABSTRACT

Spontaneous autopolyploidy, a 1.5x increase in genome size resulting from second polar body retention after fertilization, has been documented in cultured sturgeons, with the proportion of spontaneous autopolyploid progeny ranging widely among maternal families. Sturgeon farms wish to reduce the number of spontaneous autopolyploids because their progeny, when crossed with a normal ploidy parent, exhibit intermediate ploidies, resulting in reproductive abnormalities. However, there is limited knowledge of the causes of the second polar body retention in sturgeon culture. In this study, we report the results of experiments performed from 2015 to 2019 aimed at identifying the sources of spontaneous autopolyploidy in white sturgeon (*Acipenser transmontanus*) culture. In collaboration with several sturgeon farms, we tested whether post-ovulatory ageing, mechanical shock during egg de-adhesion, and the combined effects of both factors increased spontaneous autopolyploidy. To test the effect of post-ovulatory ageing, eggs were collected from females and either fertilized at the industry normative time (2–5 h post-oviposition) or stored in ovarian fluid at 15 °C for 6–8 h before fertilization. To test the effect of mechanical shock, eggs were collected, fertilized 2–5 h post-oviposition and exposed to either gentle or vigorous mixing during the 60 min de-adhesion treatment. Results from this work reveal that post-ovulatory ageing does increase the incidence of spontaneous autopolyploidy in some females, but overall the proportions produced were low (range 0–15%). Proportions of spontaneous autopolyploids in eggs exposed to vigorous mixing were also variable (1–92%) but significantly higher in 75% of the females when compared to their respective controls or gentle mixing treatments, indicating that mechanical shock during egg de-adhesion is likely the primary cause of spontaneous autopolyploidy. To our knowledge this is the first study to document mechanical shock to eggs during de-adhesion as a cause of abnormal ploidy in cultured fishes. We observed high variability in egg quality among females and a significant relationship between embryo mortality and the incidence of spontaneous autopolyploidy when eggs were exposed to mechanical shock. Repeated spawning of a female that produced a high proportion of spontaneous autopolyploids provided preliminary evidence that genetic background may influence the likelihood that a female's eggs will be prone to second polar body retention when subjected to mechanical shock. Although spontaneous autopolyploidy in sturgeon culture will likely never be eliminated entirely, we provide practical recommendations to sturgeon producers to reduce its incidence in a production setting.

## 1. Introduction

Spontaneous autopolyploidy is a 1.5x increase in genome size that occurs without a management intervention aiming to induce triploidy. Although spontaneous autopolyploids (SAs) have been reported in a wide variety of taxa, including cartilaginous fishes (Kendall et al.,

1994), bony fishes (Flajshans et al., 1993; Glover et al., 2015; Nomura et al., 2013; Zhang and Arai, 1999), amphibians (Litvinchuk and Rosanov, 2016), reptiles (Rovatsos et al., 2018), and birds (de Camargo et al., 2017), the phenomenon is typically rare. However, spontaneous autopolyploidy has been frequently documented in polyploid sturgeons (Acipenseriformes), including the Gulf sturgeon (*Acipenser oxyrinchus*

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desotoi; Blacklidge and Bidwell, 1993), kaluga (*Huso dauricus*; Zhou et al., 2011), lake sturgeon (*A. fulvescens*; Blacklidge and Bidwell, 1993), Sakhalin sturgeon (*A. mikadoi*; Havelka et al., 2017; Zhou et al., 2011), sterlet (*A. ruthenus*; Havelka et al., 2013), Siberian sturgeon (*A. baeri*; Havelka et al., 2014), and white sturgeon (*A. transmontanus*; Drauch Schreier et al., 2011). The reason for a higher incidence of spontaneous autopolyploidy in sturgeon relative to other taxa is unknown but suspected to be related to the species' already polyploid genomes (possessing ~120, ~240, or ~360 chromosomes; Fontana, 1994; Kim et al., 2005), which may be better able to cope with additional gene copies than a fully diploidized genome (Gille et al., 2015).

Thus far, spontaneous autopolyploidy has been best studied in the ancestral octoploid (8N; ~240 chromosomes) white sturgeon, a species native to North America that is grown for conservation and commercial purposes in several countries (Bronzi et al., 2019; Hildebrand et al., 2016). Spontaneous autopolyploidy has been documented in both conservation and commercial hatcheries. On average, ~10% of individuals at a facility have abnormal ploidy, although the incidence within families varies widely (Schreier et al., 2013). For example, we've observed families containing no SAs and families that contain 92% SAs (Schreier et al., unpublished data; this study). Although SA white sturgeon are viable and fertile (both sexes), they are a concern for both conservation and commercial white sturgeon production because crosses of normal (8N) and SA (12N) adults produce viable progeny with an intermediate genome size (10N) that exhibit abnormalities in gonadal development. While 10N males exhibit normal reproductive development, sexual maturity in 10N females is delayed relative to 8Ns of the same age (Schreier and Van Eenennaam, 2019). Differences in metabolic enzyme activity between 10N and both normal and SA white sturgeon have been observed, including reduced metabolic reorganization when acclimated to warmer water (Leal et al., 2018). Release of SAs into wild populations could lead to production of 10N progeny that may have an impaired ability to acclimatize to warming temperatures and also exhibit delayed maturation. Spawning SA broodstock in a commercial facility will produce 10N progeny that may perform suboptimally at warm culture temperatures and females must be reared longer to produce caviar, increasing production costs. Knowing the potential causes of spontaneous autopolyploidy would allow conservation hatcheries and sturgeon farms to reduce its incidence, protect vulnerable wild populations and maximize caviar production efficiency, respectively.

There are two potential proximate causes of spontaneous autopolyploidy in fishes: polyspermy and second polar body retention. Polyspermy, or the penetration of the egg by > 1 sperm, can lead to the transmission of two or more paternal genome copies. Although polyspermy has been documented in white sturgeon when high sperm concentrations are used for fertilization (Cherr and Clark, 1985), it is uncommon in hatcheries because milt is diluted (Van Eenennaam et al., 2004). A study by Gille et al. (2015) demonstrated that SA white sturgeon inherited two copies of the female genome, implicating retention of the second polar body during meiosis II as the proximate cause of spontaneous autopolyploidy in sturgeon. Retention of the second polar body is the same mechanism by which triploidy has been induced in sturgeon using thermal or pressure treatments applied 12–20 min post-fertilization (Flynn et al., 2006; Fopp-Bayat et al., 2007; Fopp-Bayat, 2010; Van Eenennaam et al., 1996).

Since targeted triploid induction is not currently practiced, our goal was to determine the causes of spontaneous autopolyploidy in white sturgeon hatcheries. One cause could be post-ovulatory ageing, which is considered one of the most important factors affecting egg quality (Bobe and Labbé, 2010; Migaud et al., 2013; Samarin et al., 2015). As the time between ovulation and fertilization increases, oocytes experience oxidative stress and related biochemical changes, alteration in maternal genome expression, and re-organization of cytoskeletal components necessary for successful meiotic divisions, including extrusion of the second polar body (Boulebach et al., 1989; Samarin et al., 2018;

Webb et al., 1986). Post-ovulatory ageing has been shown to increase rates of triploidization in numerous fishes (Aegerter and Jalabert, 2004; Flajshans et al., 1993; Nomura et al., 2013; Pereira dos Santos et al., 2018; Várkonyi et al., 1998), including bester sturgeon (*Huso huso* x *Acipenser ruthenus*; Omoto et al., 2005).

Sturgeon farms usually spawn more than one female on any specific day, due to the time, labor, and facility commitment required for each spawning event and because more than one female from a large captive broodstock will usually be ready for spawning induction at the same time. If 3–6 females are induced to spawn on the same day, there could be 2–3 females that begin ovulation simultaneously. Since processing each female takes about 1.5 h (collect eggs by caesarean surgery, suture the incision, egg de-adhesion; Van Eenennaam et al., 2004), the 2nd and/or 3rd female spawned may have been fully ovulated for several additional hours beyond the usual time of egg collection, which is 1–5 h after first oviposition (Van Eenennaam et al., 2004, unpublished data). Thus, our first hypothesis was that post-ovulatory ageing, due to delayed collection of eggs from an ovulated female, could impact SA production.

We also considered the techniques intentionally used to induce triploidy in a variety of aquaculture species, including chemical, pressure and thermal treatments (Piferrer et al., 2009). Chemical and pressure treatments are not applied to white sturgeon eggs in culture so these were eliminated from consideration. Because spawning induction, egg collection, fertilization, and de-adhesion (to prevent clumping) occur at controlled temperatures in both conservation and commercial facilities (Conte et al., 1988; Van Eenennaam et al., 2004), thermal shock also seemed like an unlikely cause of spontaneous autopolyploidy. Mechanical shock, although not a traditional method of inducing triploidy, was considered a possibility during the de-adhesion process, when eggs are mixed in a solution of various types of clay, bentonite, or Fuller's earth for approximately 1 h after the initial 2 min of fertilization (Conte et al., 1988; Dettlaff et al., 1993; Van Eenennaam et al., 2004) to prevent eggs from adhering to each other.

Over the decades, we have observed highly variable modes of mixing eggs during de-adhesion: from very gentle, occasional back and forth motion using a feather, or one or both hands, to continuous, and faster, back and forth, and/or swirling motions (J. Van Eenennaam and S. Doroshov, personal observations). The mechanical shock is hypothesized to be from the sudden acceleration and deceleration of eggs colliding with each other or the sides of the bowl while subjected to underwater wave action created by hand(s) or a feather. These forces could be enough to disrupt the spindle apparatus and the second polar body would be retained. Mixing by hand was initially recommended to ensure hatchery personnel could feel the de-adhesion process and if any eggs were clumping or sticking to the walls of the bowl, the clumps could be gently broken up or the eggs rolled off the sides of the bowl by hand (Conte et al., 1988). Because the period of de-adhesion corresponds to the timeframe in which second polar body retention would be induced in aquaculture, we hypothesized that the mechanical shock to the eggs from too vigorous de-adhesion could be a cause of spontaneous autopolyploidy.

In this five-year study, we tested the hypotheses that post-ovulatory ageing and mechanical shock during egg de-adhesion contribute to SA production in sturgeon hatcheries. Eggs were collected from females at collaborating sturgeon farms, subjected to post-ovulatory ageing, vigorous mixing during de-adhesion, or both, and the ploidy of resulting progeny was measured. The results of our experiments provide management recommendations that can be used to reduce the incidence of spontaneous autopolyploidy in sturgeon culture.

## 2. Methods

### 2.1. Broodstock and spawning

All ovulated eggs and milt used in these experiments were obtained from captive broodstock at collaborating sturgeon farms. Farm staff sampled eggs from each female 2–3 times during winter and spring by

biopsy (Chapman and Van Eenennaam, 2007a) to monitor individual progress through final maturation by measuring polarization index (PI), or the distance of the germinal vesicle from the animal pole divided by the egg diameter ( $n = 12$ ) along the animal-vegetal axis. Body weight was obtained from females during the last PI check, and spawning was scheduled when PI was  $\leq 0.10$  (Chapman and Van Eenennaam, 2007a). A subsample of the eggs at the final PI check and ovulated eggs at spawning were obtained from each female. PI and maximum egg diameter (animal-vegetal axis) were measured at the Center for Aquatic Biology and Aquaculture at the University of California, Davis (UCD). Measurements ( $\pm 0.001$  mm,  $n = 12$ ) were made under a dissecting microscope equipped with a camera (lucida) using a digital image-analyzing tablet (Nikon Microplan II).

To induce ovulation and spermiation, females and males received a weight-dependent total dose  $20\text{--}50\text{ }\mu\text{g kg}^{-1}$  and  $10\text{--}20\text{ }\mu\text{g kg}^{-1}$ , respectively, of mammalian gonadotropin-releasing hormone analogue (LHRHa); Des-Gly<sup>10</sup>,D-Ala<sup>6</sup>, Pro-NHET<sup>9</sup>-LHRH ethylamide acetate salt (Western Chemical, Inc. Ferndale, WA, USA). Females received a primer dose (10% of the total), followed 12 h later by the resolving 90% dose. Males received a single injection at the time of the female's first injection. Beginning approximately 16 h after the resolving injection and every hour following, farm personnel checked individual females for ovulation and recorded the time of first oviposition by observing and netting the bottom of the females' holding tanks for ovulated eggs. Time of egg fertilization was reported as hours after first oviposition. Ovulated eggs were collected by caesarean surgery and milt with 60 cc syringes, as described in Van Eenennaam et al. (2004). On farm water temperature during broodstock injections, egg collection and embryo incubation was  $15 \pm 1^\circ\text{C}$ .

Ovulated eggs (200–250 ml; approximately 5000) used for each females' experimental treatments were subsampled from the first bowl collected by farm staff, and either fertilized immediately (controls and mechanical shock treatments) or covered in ovarian fluid and kept in oxygenated bags which were placed in an ice chest with gel packs until fertilization 6–8 h later (ageing treatments). Milt from a single male was used for each females' treatments and was selected based on the criterion of having over 70–80% motile spermatozoa that were motile for at least 2 min. Milt was kept in a 250 ml Erlenmeyer flask if used for immediate fertilization or in an oxygenated bag for ageing experiments. The Erlenmeyer flasks and oxygenated bags were placed in an ice chest on wet ice until used. Milt used for the ageing treatments was rechecked, 6–8 h later, and all were verified to have a similar percent motility ( $\pm 10\%$ ) and time of motility ( $\pm 30$  s) to when it was used for the control treatments.

Fertilization was the same for all treatments. Milt was diluted 1:200 with hatchery water (10 ml:2000 ml) and immediately poured into a 30 cm diameter bowl (7 L volume) containing the ovulated eggs. The eggs and milt were very slowly mixed back and forth with a feather for 2 min, just enough to ensure all eggs were moved up from the bottom of the bowl and exposed to motile sperm. The milt-water mixture was then poured off and a mixture of 100 ml of 100-mesh Fuller's earth (Sigma-Aldrich, St. Louis, MO, USA) and 2 L of hatchery water was poured over the fertilized eggs. The de-adhesion process for all treatments lasted for 1 h, and the specifics for each experiment are given below.

#### 2.1.1. E1 Spring 2015–2016 ageing experiment

Four females were used to examine the potential effect of *in vitro* post-ovulatory ageing on the production of SA progeny. *In vitro* ageing was considered a proxy for *in vivo* ageing that might occur if there was a significant delay between ovulation and egg collection during spawning. Eggs were collected as described above in section 2.1. Controls consisted of eggs fertilized at the same time the farm staff fertilized their first bowl of production eggs, 2–5 h post-oviposition. Eggs in ageing treatments were covered in ovarian fluid and kept in oxygenated bags at  $15 \pm 1.0^\circ\text{C}$  for 6–7 additional hours before fertilization. De-adhesion of both experimental and control ageing

experiment groups was the same; eggs were moved slowly with a feather ( $\sim 3$  s to sweep along the 15 cm bowl bottom), using a continuous, back and forth motion.

#### 2.1.2. E2 Spring 2016–2019 mechanical shock experiments

Eleven females were used in twelve spawning events (one female spawned twice) to test the effect of mechanical shock during egg de-adhesion on the production of SAs. In the gentle treatment a feather was used to very slowly and occasionally ( $\sim 5$  s per sweep, once every 10 s) lift the eggs off the bottom of the bowl. In the vigorous treatment, a feather was also used but rapid ( $< 1$  s per sweep) and continuous movement of the eggs was performed with a back-and-forth motion.

#### 2.1.3. E3 Spring 2018 combined egg ageing and mechanical shock experiments

Four females were used to compare the potential combined effect of *in vitro* post-ovulatory ageing (6–7 h), followed by mechanical shock (treatments: age + gentle de-adhesion and age + vigorous de-adhesion) on the incidence of spontaneous autopolyploidy in comparison to the farm treatment of the eggs (control-not aged). Gentle and vigorous treatment procedures were the same as described above in section 2.1.2.

Because of the results of E2 (2.1.2), all farms were silting their bowls of eggs very slowly and gently during de-adhesion when E3 was performed. De-adhesion of their much larger bowls of control eggs (2 bowls per female) were with one or both hands for the 1 h, then eggs were rinsed clean of Fuller's earth and sub-sampled (approximately 100–125 ml from each bowl).

#### 2.1.4. E4 Spring 2019 combined egg ageing and mechanical shock experiments

Two females were used to compare the farm control (as described in 2.1.3), with the initial mechanical shock treatments (gentle and vigorous), and then a combination of 7–8 h post-ovulatory ageing and mechanical shock (age + gentle and age + vigorous) on the incidence of spontaneous autopolyploidy. This experiment allowed us to further examine how all the different treatments affected eggs of individual females.

### 2.2. Embryo transportation-incubation, larval rearing, and ploidy determination

Regardless of experimental treatment, after de-adhesion, eggs were handled using the same methods. The bowls were rinsed repeatedly with hatchery water until clean of the Fuller's earth, and the eggs were poured into a plastic bag containing 3 L of hatchery water. The bags were filled with oxygen, sealed, and placed into insulated ice chests with gel packs to keep the internal temperature at  $15 \pm 1.0^\circ\text{C}$ .

Transport to the UCD Putah Creek Hatchery Facility took 30–60 min, depending on the farm location. Upon arrival the bags of fertilized eggs were checked to see if the water temperature was within  $1^\circ\text{C}$  of the hatchery system ( $15 \pm 1^\circ\text{C}$ ), and if so, the eggs were immediately poured into separate McDonald hatching jars. On occasion the temperature was  $1.5\text{--}2.0^\circ\text{C}$  cooler than  $15^\circ\text{C}$  and these bags were allowed to acclimate by floating in the hatchery system water for 1 h before being poured into McDonald jars.

For the *in vitro* post-ovulatory ageing treatments, bags of ovulated eggs transported to UCD were floated in the hatchery system to maintain temperature until the end of the allotted ageing time. Bagged milt remained chilled on wet ice until fertilization. After fertilization and de-adhesion, as previously described, the Fuller's earth was rinsed clean and the eggs transferred directly into individual McDonald jars.

Embryos were sampled twice, in triplicate, from each treatment jar at the 4–8 cell (stage 6;  $\approx 5\text{--}6$  h after fertilization) to determine fertilization success, and then at neurulation (stage 22;  $\approx 60\text{--}70$  h after fertilization) to estimate percent hatch (Dettlaff et al., 1993). After hatch, which occurred at 6–7 days post-fertilization, larvae were slowly

acclimated to well water at  $18 \pm 1^\circ\text{C}$  over a period of three days. After yolk-sac depletion (9–11 days post-hatch), larvae were initially fed daily, ad libitum, using a 24 h belt feeder and a semi-moist diet (Rangen Inc, Buhl, Idaho; 45% protein, 18% fat), and at approximately 2 months post-hatch ( $\approx 10\text{ g}$ ) they were weaned onto extruded sinking classic trout diet (Skretting USA, Tooele, Utah; 40% protein, 12% fat). Incubation and rearing methods followed Van Eenennaam et al. (2004).

The proportion of SA (12N) progeny and other ploidies (normal 8N, 4 N/8N mosaics, aneuploids) in each treatment ( $n = 150$ ) was determined by using a Z2 Coulter® Particle Count and Size Analyzer (Beckman Coulter, Inc., Brea, CA), which measures the volume of red blood cell nuclei and has shown 100% agreement with results obtained using flow cytometry (Fiske et al., 2019). Fifty individuals were selected from each treatment culture tank from three locations; the upper water surface, mid-depth, and tank bottom regions, as the sturgeon were widely dispersed throughout the tank water column. Ploidy was determined from blood collected either by euthanasia with 500 mg/L buffered tricaine methanesulfonate (Tricaine-S, Western Chemical, Inc., Ferndale, WA) and tail ablation (ages 10–50 days post-hatch), or anesthesia (100 mg/L buffered Tricaine-S) and use of an insulin syringe (ages 50 days to 8 months post-hatch). Tails were ablated using a single-edge razor blade, and the drop of blood forming from the caudal vasculature was touched to the surface of a 25 mL blood cell counting cuvette containing 10 mL of Isoton II diluent and 3 drops of Zap-oglobin II lytic reagent (Beckman Coulter, Inc., Brea, CA). Fish that were anesthetized in buffered Tricaine-S were at least 5–7 g in body weight, and only a tiny drop of blood was collected via the caudal vasculature using a 31 gauge, 1/3 cc insulin syringe inserted immediately anterior to the anal fin. No anticoagulant was needed in the syringes as the drop of blood was immediately placed into the cuvette. All fish recovered from the anesthesia and no post-sampling mortalities were observed. Ploidy was measured at different ages across years because the purchase of the Coulter® counter was delayed in 2015 and in some years, larger and older individuals of verified ploidy were needed for additional experiments (Leal et al. 2018, 2019). All rearing, handling and sampling procedures were approved by the UCD Institutional Animal Care and Use Committee (protocol #19778).

### 2.3. Data analysis

All statistical analyses were conducted in R (<http://www.Rproject.org>) using the RStudio interface (v3.3.0). A Fisher's exact test was performed on numbers of fertilized eggs, neurulated embryos, and SA progeny to identify significant differences due to treatment effects within each female. Initial comparisons were made at the level of individual females rather than treatment due to high variability in egg quality and propensity to produce SA progeny among females.

All data were then tested for homogeneity of variances and normal distribution of residuals using a Levene's test and Shapiro-Wilk test, respectively. For percentage values, data were arcsine transformed prior to these analyses. A multiple linear regression was performed to assess the effect of egg quality and treatment (control, gentle, vigorous, age + gentle, age + vigorous) on proportion of SAs. The 2015–2016 (Table 1) aged treatments were included in the age + gentle group, as these eggs experienced gentle de-adhesion. The linear model included percent SA as the dependent variable with PI, embryo mortality (drop in the proportion of viable eggs between 4 and -8 cell and neurulation stages within each female x treatment group; FertNeurl), days to injection after last PI check (Days), and treatment as explanatory variables:

$$\begin{aligned} \%SA = & PI + FertNeurl + Days + Treatment + FertNeurl \times Treatment \\ & + Days \times Treatment + FertNeurl \times Days \\ & + FertNeurl \times Days \times Treatment + E \end{aligned}$$

Significant effects of treatment, PI and embryo mortality were assessed using an ANOVA. Significant differences between treatments

were determined using Tukey's post hoc test ( $\alpha = 0.05$ ). All data are presented as mean  $\pm$  standard deviation.

## 3. Results

### 3.1. Broodstock and spawning

Body weight of the 21 female broodstock used in this study ranged from 30 to 90 kg ( $54.3 \pm 15.5\text{ kg}$ , mean  $\pm$  SD), and mean egg diameter varied from 3.38 to 4.18 mm ( $3.59 \pm 0.19\text{ mm}$ ). PI at last check, prior to spawning induction, ranged from  $0.044 \pm 0.012$  to  $0.101 \pm 0.011$ . All females received their first hormone injection within 3–15 days of the last PI check, with the exception of one female that was injected at day 46, due to the farm waiting for a 2nd female also to be ready for spawning induction.

#### 3.1.1. E1 Spring 2015–2016 ageing experiment

All four females were induced to spawn 14 days after their last PI check ( $0.065\text{--}0.075$ ) and had good quality eggs, as indicated by high fertility ( $\geq 89\%$ ) and neurulation rates ( $\geq 79\%$ ) in the control treatments (Table 1). The *in vitro* post-ovulatory ageing of 7 h significantly reduced mean fertilization and neurulation rates in only one female (O39), indicating a potential impact of ageing, but there was no incidence of spontaneous autopolyploidy (Table 1). Only one of four females (Y39) showed a significant increase in spontaneous autopolyploidy (14.7%) after post-ovulatory ageing of eggs (Table 1). The elevated levels of spontaneous autopolyploidy for that female, also seen in the control (5.3%), could be associated with an even longer ageing time than designed because the actual time of first oviposition was missed for Y39, so the actual time of *in vivo* ageing, and subsequent time to fertilization, was estimated.

We learned after the spawning event that female Y40 was actually a SA and when crossed with a normal 8N male produced 10N progeny. Theoretically, this female could have produced 16N SAs but did not (Table 1). We concluded from female Y39 that *in vitro* post-ovulatory ageing may lead to spontaneous autopolyploidy in sturgeon culture but the overall low proportions in the other aged treatments suggests it is likely not the leading cause.

#### 3.1.2. E2 Spring 2016–2019 mechanical shock experiments

We observed no significant differences in mean fertilization or neurulation success between gentle and vigorous treatments in all but one female (Table 2). The exception was R25, but this female's embryo mortality was similar for both treatments (6–8%), and we are almost certain that the bowl of eggs that received the vigorous treatment was contaminated by some splashing water prior to fertilization, activating some of the eggs, which resulted in the 34% lower initial fertility rate compared to the gentle treatment (Table 2).

There were several females with high embryo mortality rates after fertilization, ranging from a 13–17% mortality (W08, R01, R14, W39) in both the gentle and vigorous treatments, indicating potentially lower quality eggs (Table 2). Female R01, which had treatments with the lowest fertility (50%) and neurulation success (37, 36%), also had the lowest PI (0.044), possibly indicating overripe eggs that when treated very gently produced no SAs, but when vigorously handled produced 23% SAs.

For 75% of females, the mechanical shock from vigorous mixing during de-adhesion caused a significant increase in the proportion of SA progeny. The proportion of SA progeny in the vigorous treatment was highly variable between females, and ranged from 0.7 to 80.0% (mean 23.9%) while in the gentle treatment, the proportion of SAs ranged from 0.0 to 52.0% (mean 4.9%) (Table 2). Female R14, when spawned in 2017, produced a high proportion of SA progeny in both the gentle and vigorous treatments (52.0%, 80.0% respectively, Table 2). When this individual was spawned again in 2019 to see if she would replicate a similarly high number of SAs, she produced a lower proportion in the



**Table 1**

Results of *in vitro* post-ovulatory ageing experiments conducted during spring 2015 and 2016. PI is polarization index (n = 12). Days to 1st Inj refers to the time between the last PI check and first hormone injection, and Time to Fert is the time between first observation of oviposition and fertilization. Percent SA was based on Coulter counter analysis (n = 150). Fertility and neurulation success are for triplicate sub-samples, and all data are means  $\pm$  standard deviation. An asterisk denotes significant differences between treatments within a female (P < 0.05).

Year/ID	PI	Days to 1st Inj	Time to Fert (hr)	Treatment	Fert (%)	Neurl (%)	SA (%)
2015/I00	0.068 $\pm$ 0.012	14	4	Control	95 $\pm$ 2	89 $\pm$ 1	0.0
			10	Aged 6 h	90 $\pm$ 2	83 $\pm$ 2	0.7
2015/Y40	0.065 $\pm$ 0.012	14	3	Control	93 $\pm$ 3	92 $\pm$ 2	0.0 <sup>a</sup>
			10	Aged 7 h	90 $\pm$ 1	85 $\pm$ 6	0.0 <sup>a</sup>
2016/O39	0.073 $\pm$ 0.024	14	$\approx$ 4 <sup>b</sup>	Control	89 $\pm$ 2*	86 $\pm$ 1*	0.0
			$\approx$ 11 <sup>b</sup>	Aged 7 h	68 $\pm$ 5*	69 $\pm$ 1*	0.0
2016/Y39	0.075 $\pm$ 0.020	14	$\approx$ 5 <sup>b</sup>	Control	94 $\pm$ 2	79 $\pm$ 1	5.3*
			$\approx$ 12 <sup>b</sup>	Aged 7 h	91 $\pm$ 1	79 $\pm$ 2	14.7*

<sup>a</sup> Y40 was a 12 N crossed with an 8 N male and all progeny were 10 N.

<sup>b</sup> At first egg check, hundreds (Y39) to thousands (O39) of eggs were found, so actual time to fertilization is estimated.

gentle (4.0%) but a similarly high proportion in the vigorous treatment (64.0%).

### 3.1.3. E3 Spring 2018 combined egg ageing and mechanical shock experiments

Fertility and neurulation success for all controls and treatments was above 70%, except for female I15 neurulation (45–63%, Table 3). All aged treatments had similar fertility rates compared to the farm control, but two females (I19, I15) had significantly lower neurulation rates, indicating a higher embryo mortality with ageing (Table 3).

The proportion of SAs was the lowest in the farm controls (0.0–1.3%). In the age + gentle treatments, spontaneous autopolyploidy ranged from 0.0 to 6.0%, with females I15 and O99 having significantly higher proportions than the controls, indicating an effect of ageing. The combined effect of ageing + vigorous handling was seen in three of the four females, and they had the highest incidence (6–21.3%) of SAs in this treatment (Table 3). The highest incidence of spontaneous autopolyploidy (21.3%) was in the age + vigorous treatment for female O99 which also had the lowest PI (0.048). The next

highest incidence of spontaneous autopolyploidy (4.0 and 9.3%) was for female I15 which also had the highest embryo mortality in the aged treatments (24–26%). Female W85 appears to have had high quality eggs showing no reduction in fertility or neurulation success with ageing, and no impact of vigorous handling in terms of SA production (0.0–1.3%). However, in this age + vigorous treatment we also identified for the first time some aneuploids (1.3%) and mosaics (4 N/8 N, 3.3%) (Table 3).

### 3.1.4. E4 Spring 2019 combined egg ageing and mechanical shock experiments

When age + gentle and age + vigorous treatments were compared to gentle and vigorous treatments, we found that *in vitro* post-ovulatory ageing increased the proportion of SAs beyond the gentle and vigorous treatments in female I32 (Table 4). This female also produced 4% 4 N/8 N mosaic individuals in the age + vigorous treatment (Table 4). However, in female 008, we found that post-ovulatory ageing actually increased egg quality. This female had the highest rate of embryo mortality in this study, but both fertility and neurulation were higher in

**Table 2**

Results of mechanical shock experiments with post-fertilized eggs for females (ID) during spring 2016–2019. PI is polarization index (n = 12). Days to 1st Inj refers to the time between the last PI check and first hormone injection, and Time to Fert is the time between first observation of oviposition and fertilization. Percent SA was based on Coulter counter analysis (n = 150). Fertility and neurulation success are for triplicate sub-samples, and all data are means  $\pm$  standard deviation. An asterisk denotes significant differences between treatments within a female (P < 0.05).

Year/ID	PI	Days to 1st Inj	Time to Fert (hr)	Treatment	Fert (%)	Neurl (%)	SA (%)
2016/A54	0.084 $\pm$ 0.010	11	$\approx$ 4 <sup>a</sup>	Gentle	89 $\pm$ 1	86 $\pm$ 4	0.0
				Vigorous	90 $\pm$ 1	88 $\pm$ 2	1.3
2016/O62	0.070 $\pm$ 0.012	46	$\approx$ 7 <sup>a</sup>	Gentle	95 $\pm$ 2	90 $\pm$ 1	0.7*
				Vigorous	96 $\pm$ 2	90 $\pm$ 2	43.3*
2017/W08	0.080 $\pm$ 0.011	13	2	Gentle	80 $\pm$ 2	64 $\pm$ 5	1.3*
				Vigorous	82 $\pm$ 3	65 $\pm$ 5	44.0*
2017/R01	0.044 $\pm$ 0.012	15	2	Gentle	50 $\pm$ 1	37 $\pm$ 2	0.0*
				Vigorous	50 $\pm$ 1	36 $\pm$ 1	23.0*
2017/R14	0.073 $\pm$ 0.012	15	3	Gentle	90 $\pm$ 2	75 $\pm$ 2	52.0
				Vigorous	90 $\pm$ 2	75 $\pm$ 3	80.0
2018/W39	0.101 $\pm$ 0.011	12	2	Gentle	97 $\pm$ 1	81 $\pm$ 3	0.7*
				Vigorous	95 $\pm$ 3	81 $\pm$ 3	6.0*
2018/R25	0.058 $\pm$ 0.009	6	2	Gentle	85 $\pm$ 2*	77 $\pm$ 1*	0.0*
				Vigorous	51 $\pm$ 3*	45 $\pm$ 1*	4.0*
2018/I04	0.094 $\pm$ 0.013	14	2	Gentle	89 $\pm$ 1	82 $\pm$ 3	0.0
				Vigorous	91 $\pm$ 4	81 $\pm$ 2	0.7
2018/W34	0.064 $\pm$ 0.016	7	2	Gentle	90 $\pm$ 1	89 $\pm$ 2	0.0*
				Vigorous	91 $\pm$ 2	90 $\pm$ 3	4.0*
2019/W31	0.055 $\pm$ 0.011	7	2	Gentle	64 $\pm$ 4	66 $\pm$ 5	0.0*
				Vigorous	65 $\pm$ 2	66 $\pm$ 9	10.7*
2019/A01	0.058 $\pm$ 0.010	3	3	Gentle	85 $\pm$ 1	84 $\pm$ 2	0.0*
				Vigorous	85 $\pm$ 1	83 $\pm$ 2	6.0*
2019/R14	0.066 $\pm$ 0.019	5	2	Gentle	83 $\pm$ 3	84 $\pm$ 3	4.0*
				Vigorous	84 $\pm$ 3	82 $\pm$ 3	64.0*

<sup>a</sup> At first egg check, hundreds of eggs were found, so time to fertilization is estimated.

**Table 3**

Combined *in vitro* post-ovulatory ageing and mechanical shock experiments, compared with a non-aged farm control, for females (ID) during spring 2018. PI is polarization index ( $n = 12$ ). Days to 1st Inj refers to the time between the last PI check and first hormone injection, and Time to Fert is the time between first observation of oviposition and fertilization. Percent SA was based on Coulter counter analysis ( $n = 150$ ). Fertility and neurulation success are for triplicate sub-samples, and all data are means  $\pm$  standard deviation. Differences in letters denotes significant differences between treatment means within a female ( $P < 0.05$ ).

Year/ID	PI	Days to 1st Inj	Time to Fert (hr)	Treatment	Fert (%)	Neurl (%)	SA (%)
2018/I19	0.090 $\pm$ 0.014	10	2	Farm Control	96 $\pm$ 1 <sup>a</sup>	90 $\pm$ 2 <sup>a</sup>	0.7 <sup>a</sup>
			9	Aged 7 h + Gentle	94 $\pm$ 1 <sup>ab</sup>	86 $\pm$ 1 <sup>b</sup>	0.7 <sup>a</sup>
			9	Aged 7 h + Vigorous	92 $\pm$ 1 <sup>b</sup>	86 $\pm$ 2 <sup>b</sup>	6.0 <sup>b</sup>
2018/I15	0.081 $\pm$ 0.010	12	3	Farm Control	79 $\pm$ 4 <sup>a</sup>	63 $\pm$ 3 <sup>a</sup>	0.0 <sup>a</sup>
			9	Aged 6 h + Gentle	73 $\pm$ 3 <sup>ab</sup>	49 $\pm$ 3 <sup>b</sup>	4.0 <sup>b</sup>
			9	Aged 6 h + Vigorous	71 $\pm$ 1 <sup>b</sup>	45 $\pm$ 3 <sup>b</sup>	9.3 <sup>c</sup>
2018/W85	0.077 $\pm$ 0.010	14	4	Farm Control	85 $\pm$ 3 <sup>a</sup>	74 $\pm$ 2 <sup>a</sup>	1.3 <sup>a</sup>
			11	Aged 7 h + Gentle	86 $\pm$ 3 <sup>a</sup>	75 $\pm$ 2 <sup>a</sup>	0.0 <sup>a</sup>
			11	Aged 7 h + Vigorous	87 $\pm$ 3 <sup>a</sup>	74 $\pm$ 1 <sup>a</sup>	1.3 <sup>aa</sup>
2018/O99	0.048 $\pm$ 0.009	14	5	Farm Control	92 $\pm$ 3 <sup>a</sup>	88 $\pm$ 1 <sup>a</sup>	0.0 <sup>a</sup>
			11	Aged 6 h + Gentle	90 $\pm$ 4 <sup>a</sup>	86 $\pm$ 1 <sup>a</sup>	6.0 <sup>b</sup>
			11	Aged 6hr + Vigorous	89 $\pm$ 1 <sup>a</sup>	88 $\pm$ 1 <sup>a</sup>	21.3 <sup>c</sup>

<sup>aX</sup>In addition 1.3% aneuploids ( $\approx 10$  N) and 3.3% 4 N/8 N mosaics were detected.

the aged groups and the proportion of SAs was lower (Table 4). The age + gentle and age + vigorous treatments in 008 had a drop in spontaneous autopolyploidy from 12.7% and 92% to 5.3% and 20.7%, respectively (Table 4).

### 3.1.5. Summary analyses

The ANOVA revealed a significant effect of treatment ( $p < 0.001$ ) on the proportion of SAs produced across experiments. Vigorous mixing of eggs during de-adhesion resulted in the highest percentage of SAs ( $27 \pm 32\%$ ; mean  $\pm$  SD) and was significantly higher than the control, gentle, and age + gentle treatments ( $p < 0.05$ ) (Fig. 1). Eggs that were aged and mixed vigorously during de-adhesion produced a higher proportion of SAs ( $13 \pm 8\%$ ) relative to the controls ( $2 \pm 4\%$ ), gentle ( $5 \pm 14\%$ ), and age + gentle ( $4 \pm 5\%$ ) treatments, but the difference was not significant ( $p > 0.05$ ), due to the high standard deviations.

The multiple linear regression found a positive correlation between the proportion of SAs produced and all variables examined ( $R^2 = 0.704$ ). While the final PI determination and days to injection did not have a significant effect on percent SA ( $p = 0.607$  and  $0.098$ , respectively), we found that embryo mortality (drop in the proportion of viable eggs between 4-8 cell and neurulation stages) indicating potentially lower quality eggs significantly affected the percent of SA progeny ( $p = 1.84 \times 10^{-5}$ ), and this effect depended on treatment ( $p = 0.009$ ), with the higher percentage of SA progeny coming from the vigorous treatments (Fig. 2). The vigorous treatments showed the greatest increase in percent SA per percent drop in viable eggs. The estimate for the slope of the vigorous line was  $56.9 \pm 19.3\%$ , relative to  $0.1 \pm 18.9\%$ ,  $14.5 \pm 19.7\%$ , and  $27.4 \pm 20.1\%$  for the gentle, age + gentle, and age + vigorous treatments, respectively (Fig. 2).

**Table 4**

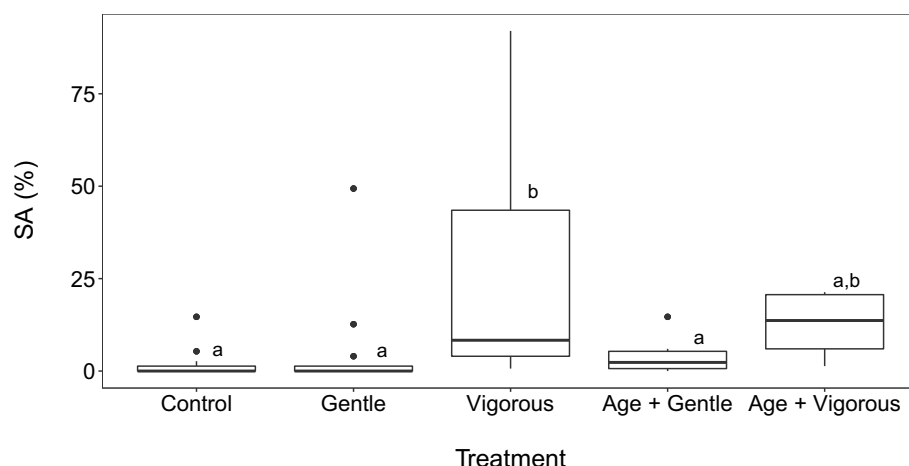
Combined *in vitro* post-ovulatory ageing and mechanical shock experiments for females (ID) compared with a farm control during spring 2019. PI is polarization index ( $n = 12$ ). Days to 1st Inj refers to the time between the last PI check and first hormone injection, and Time to Fert is the time between first observation of oviposition and fertilization. Percent SA was based on Coulter counter analysis ( $n = 150$ ). Fertility and neurulation success are for triplicate sub-samples, and all data are means  $\pm$  standard deviation. Differences in letters denotes significant differences between the treatment means within a female ( $P < 0.05$ ).

Year/ID	PI	Days to 1st Inj	Time to Fert (hr)	Treatment	Fert (%)	Neurl (%)	SA (%)
2019/I32	0.069 $\pm$ 0.011	13	3	Farm Control	58 $\pm$ 5 <sup>a</sup>	47 $\pm$ 2 <sup>a</sup>	0.7 <sup>a</sup>
			3	Gentle	59 $\pm$ 8 <sup>a</sup>	46 $\pm$ 4 <sup>a</sup>	0.0 <sup>a</sup>
			3	Vigorous	59 $\pm$ 3 <sup>a</sup>	45 $\pm$ 4 <sup>a</sup>	2.7 <sup>a</sup>
			11	Aged 8 h + Gentle	60 $\pm$ 1 <sup>a</sup>	46 $\pm$ 4 <sup>a</sup>	5.3 <sup>b</sup>
			11	Aged 8 h + Vigorous	59 $\pm$ 3 <sup>a</sup>	47 $\pm$ 4 <sup>a</sup>	18.0 <sup>ca</sup>
2019/008	0.085 $\pm$ 0.011	13	2	Farm Control	71 $\pm$ 1 <sup>a</sup>	12 $\pm$ 2 <sup>a</sup>	15.0 <sup>a</sup>
			2	Gentle	70 $\pm$ 1 <sup>a</sup>	11 $\pm$ 1 <sup>a</sup>	12.7 <sup>a</sup>
			2	Vigorous	69 $\pm$ 2 <sup>a</sup>	11 $\pm$ 2 <sup>a</sup>	92.0 <sup>b</sup>
			9	Aged 7 h + Gentle	85 $\pm$ 3 <sup>b</sup>	21 $\pm$ 1 <sup>b</sup>	5.3 <sup>c</sup>
			9	Aged 7 h + Vigorous	87 $\pm$ 3 <sup>b</sup>	19 $\pm$ 3 <sup>b</sup>	20.7 <sup>a</sup>

<sup>Y</sup>In addition 4% 4 N/8 N mosaic ploidies were detected.

## 4. Discussion

Although directed shocks to eggs such as thermal, hydrostatic, chemical and electrical are well known to cause triploidy in cultured fishes (Pandian and Koteeswaran, 1998; Piferrer et al., 2009), this study documents a novel mechanism by which white sturgeon spontaneous autopolyploids can be produced. During egg de-adhesion, continuous and vigorous mixing results in frequent collisions between eggs, the sides of the bowl and the mixing tool (feather or hands), which apparently has enough force to disrupt the spindle apparatus and result in the retention of the 2nd polar body, similar to directed shocks to induce triploidy. Our results suggest that both *in vitro* post-ovulatory ageing (Table 1) and mechanical shock during egg de-adhesion (Table 2) can contribute to spontaneous autopolyploidy in white sturgeon, but mechanical shock has a greater effect on the incidence of spontaneous autopolyploidy than post-ovulatory ageing. The proportions of SAs in eggs exposed to vigorous mixing during de-adhesion reached 92% while the greatest proportion produced by post-ovulatory ageing alone was 14.7%. Cumulative effects of post-ovulatory ageing and mechanical shock were observed in several females, where post-ovulatory ageing coupled with mechanical shock significantly increased the number of SAs relative to the control and gentle treatments (Tables 3 and 4). Future studies should focus on measuring both egg velocity during de-adhesion and the impact forces, as this has implications for both hatcheries and natural spawning stocks. Different hatcheries may use different de-adhesion techniques, such as aeration of eggs and Fuller's earth in a large conical vessel. Drifting eggs in a riverine environment could potentially adhere to substrate in a high velocity area or if not adhered, could tumble across substrate with variable force impacts, resulting in spontaneous autopolyploidy.



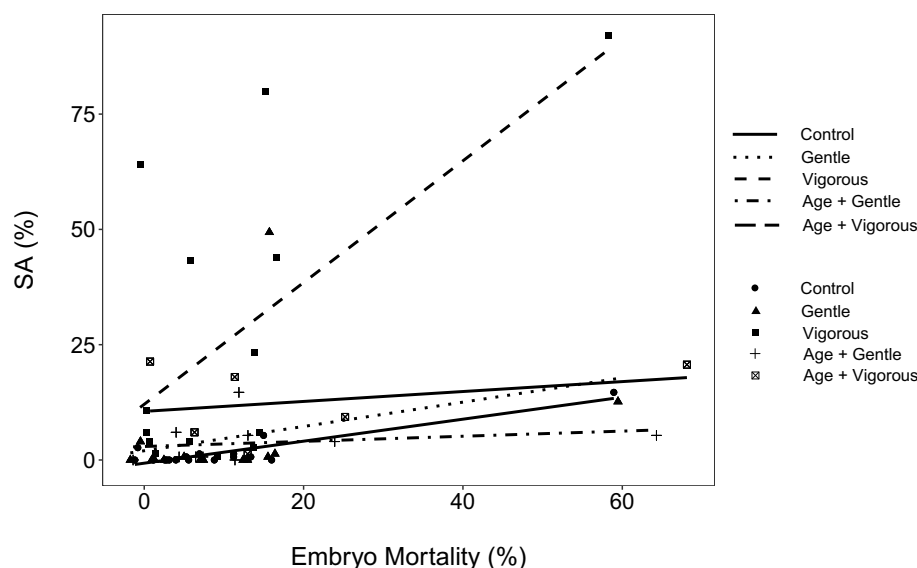
**Fig. 1.** Boxplot illustrating the proportion of spontaneous autopolyploids produced by each treatment (among females). Each box represents the interquartile range (first through third quartile). Vertical lines above and below each box represent the maximum and minimum values, respectively. Different letters indicate significant differences between treatment groups ( $P < 0.001$ ). SA = spontaneous autopolyploids. ( $n = 17$  for controls,  $n = 14$  for gentle treatment,  $n = 14$  for vigorous treatment,  $n = 10$  for age + gentle treatment and  $n = 6$  for age + vigorous treatment).

This study revealed the general resilience of white sturgeon eggs to *in vitro* post-ovulatory ageing relative to other sturgeon species. Other studies examining the effects of ageing found decreases in egg quality and reduced fertilization and embryo viability after shorter ageing times than used in this study. Significant reductions in fertilization and hatching rates were observed after 2–4 h in sterlet, 6 h in Siberian and 6 h in Persian (*A. persicus*) sturgeons (Gisbert and Williot, 2002; Hajirezaee and Niksirat, 2009). We found significant reductions in fertilization and/or neurulation rates in only three out of ten females (O39-Table 1, I19 and I15-Table 3) after 6–7 h of post-ovulatory ageing, while some females' eggs were aged 8 h without any significant reductions in viability, relative to controls. In addition, storage times can be extended based on temperature, as ovulated eggs of sterlet retained their good quality when stored at 7° and 11 °C for up to 10 h (Linhart et al., 2016), but egg quality was reduced after 5 h storage at 19 °C and 7.5 h storage at 15 °C. It should also be noted that *in vitro* egg storage is not the same as *in vivo* ageing. *In vitro* storage of eggs in ovarian fluid seems to result in a more rapid decrease of egg fertility compared to *in vivo* egg storage (Samarin et al., 2015) and Williot et al. (1991) reported Siberian sturgeon eggs retained in the abdominal cavity had high fertilization rates between 4 and 14 h after the onset of ovulation.

Although the effect of post-ovulatory ageing on spontaneous autopolyploidy has been studied in several derived fishes such as tench (*Tinca tinca*; Flajshans et al., 1993), European catfish (*Silurus glanis*; Várkonyi et al., 1998), rainbow trout (*Oncorhynchus mykiss*; Aegerter

and Jalabert, 2004), Japanese eel (*Anguilla japonica*; Nomura et al., 2013), and Northern pike (*Esox lucius*; Samarin et al., 2016), only one other study has examined it in sturgeon. Omoto et al. (2005) found that better sturgeon females that took longer to ovulate after hormone injection tended to produce SA progeny. These authors hypothesized that *in vivo* ageing, leading to lower quality, overripe eggs, was the cause of spontaneous autopolyploidy from late-ovulating female bester.

Detlaff and Goncharov (2002) noted that the potential hatching rate of sturgeon eggs, if already compromised by an improper collection time or by unfavorable environmental conditions during ovulation, could further decline due to harsh handling during de-adhesion. In this study, we did not observe any significant decrease in fertilization or neurulation rates, or any difference in embryo mortality, between the gentle and vigorous treatments. We did observe some significant increases in production of SAs with mechanical shock in females with both high fertilization and neurulation rates ( $> 80\%$ , Table 2), suggesting that vigorous handling can still cause some level of spontaneous autopolyploidy (1.3–6.0%) even in generally high quality eggs. It should be noted that not all ovulated eggs collected from a female will be the same quality, as ovulation does not take place simultaneously. Ovulation usually begins in the lower regions of the ovary and gradually spreads to the upper regions (Detlaff et al., 1993), which means some ovulated eggs collected at any given time could include a proportion of overripe eggs that are most sensitive to shocking and becoming SAs.



**Fig. 2.** Plot depicting the relationship between embryo mortality (difference between fertilization and neurulation rates) and the proportion of spontaneous autopolyploids (SAs) produced, by treatment. ( $R^2 = 0.596$ ;  $n = 17$  for controls,  $n = 14$  for gentle treatment,  $n = 14$  for vigorous treatment,  $n = 10$  for age + gentle treatment and  $n = 6$  for age + vigorous treatment).



Our multiple linear regression suggested that the rate of spontaneous autopolyploidy was significantly associated with embryo mortality, a general indicator of low egg quality, depending on treatment. Indeed, eggs from females with high embryo mortality ( $> 10\%$ ) had the highest proportions of spontaneous autopolyploidy in the vigorous treatment; however, if the same eggs are treated gently, the proportion of SAs is significantly lower (Table 2). Apparently, marginal quality eggs are more sensitive to the vigorous mixing resulting in the higher levels of spontaneous autopolyploidy.

One known determinant of egg quality is stage of final maturity (as measured by PI) at the time of induced ovulation, which varied among females. Final maturation in white sturgeon occurs over several months and is monitored by periodic sampling of ovarian follicles to determine their PI. Once the PI is at the appropriate stage, the female can be induced to ovulate (Chapman and Van Eenennaam, 2007a; Dettlaff et al., 1993). PI is even more successful in predicting ovulation success of high-quality eggs when combined with a maturational competence assay. Maturational competence, estimated by an *in vitro* oocyte assay with progesterone (Chapman and Van Eenennaam, 2007b) or 17  $\alpha$ -hydroxyprogesterone (Omoto et al., 2005) has been used for selecting female sturgeon for breeding. A higher incidence of spontaneous autopolyploidy was detected in progeny groups from better females showing a lower percent germinal vesicle breakdown in a maturational competence assay (Omoto et al., 2005). Thorgaard and Gall (1979) suggested that the stage of egg maturation is important in the production of triploid salmonids. In sturgeon, a PI that is  $\leq 0.04$ – $0.05$  may be considered overripe (Chebanov and Galich, 2011; Williot and Chebanov, 2018), increasing the chance of SA production. Only two females in this study were spawned with PI  $\leq 0.05$  (R01, Table 2; O99, Table 3), and both had high proportions of SA progeny (23 and 21%, respectively) in the vigorous treatment. A third female, O62 (Table 2), had a final PI of 0.07 but was not injected until 46 days later, 31 days longer than any other female, and undoubtedly had a PI  $< 0.05$  at the day of injection and produced 43% SAs. We did not find a significant statistical relationship between PI and proportion of SAs produced due to the high variability between females and the confounding effects of variable days to first injection. Since individual females' rate of final maturation and the change in PI over time is highly variable (Webb et al., 1999, 2001, 2002), the determination of a more accurate relationship between PI and SA production would require a future study when females are injected on the same day the final PI is determined.

Female 008 was unique to this study as she produced low quality eggs (11–21% neurulation), which improved in quality after ageing (Table 4). This was likely due to the ovulation of oocytes that had not completed maturation and the *in vitro* ageing allowed time for some portion of these eggs to complete final maturation. Oocyte final maturation and germinal vesicle breakdown is normally followed by ovulation; however, under unfavorable conditions, these processes can be desynchronized or completely inhibited (Dettlaff et al., 1993).

Stage of maturation at time of injection and treatment effects alone do not explain all the variability in fertilization, neurulation, and spontaneous autopolyploidy observed in this study. For example, female W8 was spawned at an optimal PI of 0.08 and her injection was within 14 days, but 44% of her progeny were SAs in the vigorous treatment (Table 2). This female was in a chilled recirculating system with three other females which were all atretic (soft-breaking eggs) at the last PI check due to low flows, crowding, and potential marginal dissolved oxygen levels (50–60% saturation). We believe W8 was in the earliest stages of atresia, which would have resulted in lower quality eggs and thus the high embryo mortality and high incidence of spontaneous autopolyploidy.

Another potential contributor to inter-female variation is a genetic propensity to produce SAs. Others have shown a genetic component to egg quality (Brooks et al., 1997) and Cherfas et al. (1994) suggested a genetic propensity to produce SAs in common carp (*Cyprinus carpio*). The opportunity to spawn R14 in both 2017 and 2019 allowed us to preliminarily investigate the hypothesis that certain females have a genetic predisposition to produce SAs (Table 2). In 2019, we observed fewer SAs

produced by R14 in the gentle group than in 2017 (4% vs 52%, respectively) but a similarly high proportion in the vigorous group (80% vs 64%, respectively). Interestingly, there was less embryo mortality in the 2019 spawn, suggesting R14's eggs were of higher quality, and could explain why fewer SAs were produced in the gentle group that year.

## 5. Management recommendations

Our findings can be used to develop recommendations for white sturgeon producers to reduce the incidence of spontaneous autopolyploidy. To maximize egg quality, a female should be induced to ovulate when PI is between 0.06 and 0.10 and be given the first injection within 7–14 days of the last PI check, when held at water temperatures at or below 15 °C. If multiple females will be injected for a spawning event, staggering the injection schedule 1–2 h for each female will minimize the likelihood of 2–3 females ovulating at the same time. Although *in vitro* post-ovulatory ageing should not occur in a production setting, delays in egg collection from females that ovulate simultaneously with others may lead to *in vivo* post-ovulatory ageing. Once the first oviposition for a female is detected, ovulated eggs should be collected within 1–4 h, fertilized in a timely manner (within 20–30 min), and most importantly the de-adhesion process should be very slow and gentle as to avoid mechanical shock of the eggs. Farm controls from E3 (2.1.3) and E4 (2.1.4) show that when farms follow these recommendations, they can produce low proportions of SAs similar to our gentle treatments (Tables 3 and 4).

Although these measures to minimize post-ovulatory ageing and mechanical shock during de-adhesion should benefit all sturgeon production programs, spontaneous autopolyploidy may not be eliminated entirely. Future studies should be directed toward examining the maternal influences (genetic and phenotypic) that make an individual female's eggs more or less prone to SA production. California white sturgeon farms, with an accelerated female sexual maturation rate of 7–8 years, provide an opportunity to investigate heritability of the propensity to produce SA progeny. The relationship between PI and SA production is another avenue of continued research, as this relationship may be species or population specific (e.g. Klamath River green sturgeon; Van Eenennaam et al., 2006), or depend on whether they are domestic broodstock versus wild caught.

Investigating the causes of spontaneous autopolyploidy is particularly relevant to culture programs for sturgeons possessing 120 chromosomes (ploidy group A), such as the Atlantic sturgeon (*A. oxyrinchus* spp.), sterlet, beluga (*Huso huso*) and pallid sturgeon (*Scaphirhynchus albus*). Because the group A sturgeon have genomes that are largely diploidized (Ludwig et al., 2001), we expect their SAs to exhibit impaired female reproductive development similar to the triploids of other predominantly diploid fishes (Benfey, 2015; Havelka et al., 2016; Lincoln and Scott, 1983; Varadaraj and Pandian, 1990). Therefore, SAs from group A species may themselves be unable to contribute to wild populations or caviar production.

Future research should seek to document and compare the effects of spontaneous autopolyploidy on sturgeon species of all three ploidy levels. Broadening our knowledge on the causes and extent of spontaneous autopolyploidy in sturgeon culture will better equip hatcheries to optimize their culture operations and procedures as well as reduce ecological and economic costs associated with reproductively impaired fish in conservation and commercial aquaculture programs.

## Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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